Proc. Nat. Acad. Sci. USA
Vol. 70, No. 7, pp. 1950–1954, July 1973

Crossing-Over Between X and Y Chromosomes During Ribosomal DNA Magnification in *Drosophila melanogaster*

(genetic recombination/frequency of crossing-over/circularization/integration)

F. RITOSSA

Institute of Genetics, University of Bari, Bari, Italy

Communicated by Curt Stern, March 9, 1973

ABSTRACT **In genetic combinations undergoing ribosomal DNA magnification, the frequency of crossing-over between X and Y chromosomes is increased at least 20-fold above that in similar combinations not undergoing magnification. Such recombination occurs at the ribosomal DNA level. The data are consistent with a model where extra copies of ribosomal DNA are formed which, after circularization, are integrated into the chromosome.**

Mutant *bobbed* loci (*bb*) typically carry subnormal numbers of ribosomal RNA genes (rDNA) (1). In males that have an extreme *bobbed* phenotype, magnification takes place; that is, the amount of rDNA is rapidly adjusted to normal (2). Magnification is evidenced in the progeny of such *bobbed* males. A model has been proposed for the first steps of the process (3). According to this model, extra copies of rDNA are formed in all cells of *bobbed* males. After circularization, the copies can be integrated into the chromosome only in the germ line. The first step of magnification might occur as in Fig. 1. In males with two *bb* loci, integration of rDNA copies is not necessarily restricted to the *bb* locus from which they originated (3). We expected, therefore, that some integration events might involve both *bb* loci simultaneously. Such double events (Fig. 2) would lead to recombination of outside markers.

It will be shown in this paper that X–Y exchanges at the rDNA level are at least 20 times more frequent during magnification than in similar genetic combinations not undergoing magnification.

MATERIALS AND METHODS

Stocks from the Bowling Green list included *w sn/Ybb*$^-$ & *C(1)RM,y v f/Ybb*$^-$, and sc^8 chromosomes from which *In(1)sc*^{8}, *bb*$^+$ *cv/B*^{s}*Y* & *C(1)DX,y f/B*^{s}*Y* and *In(1)sc*^{8}*,bb*^{1} *cv/B*^{s}*Y* & *C(1)DX,y f/B*^{s}*Y* were selected in our laboratory. The *Ybb* chromosome of *X*(Canton S)*/Ybb* & *C(1)RM,y*^{2} *(su)w*^{a} *w*^{a} was a gift of Dr. J. Barr in 1968. *In(1)sc*^{4L,8R}*,y cv* has a complete deletion of *bb*, sometimes indicated as *bb*^{0}. It is kept as *In(1)sc*^{4L,8R}*,y cv/B*^{s}*Y* & *C(1)DX,y f/B*^{s}*Y* (the original Oak Ridge stock), and as *In(1)sc*^{4L,8R}*/B*^{s}*Y* & *In(1)sc*^{4L,8R}*/In(1)sc*^{4L,8R}*/B*^{s}*Y*. The latter stock irregularly accumulates extra Y chromosomes. A *bb*^{1} X chromosome selected from *y w* is kept as *y w bb*^{1}*/B*^{s}*Y* & *y w bb*^{1}*/y w bb*^{1}*/B*^{s}*Y*. Homozygous *XY*^{L}·*Y*^{S} (108-9 Parker), *y*^{2} *su(w*^{a}*)* *w*^{a} and *Y*^{S}*X*·*Y*^{L}, *In(1)EN+dl49,Y*^{S} *car f v y*·*Y*^{L} & *C(1)RM y*^{2} *su(w*^{a}*)* *w*^{a} are from the University of Rome collection.

Reciprocal X–Y recombinants out of *In(1)sc*^{8}*/Y* are indicated as *y*$^+$ *sc*^{8} c.o. (crossover) *Y* and *sc*^{8} c.o. *X* elements (9). The terms "rDNA,""*bb* locus," and "rRNA genes" are used interchangeably (1).

Flies were reared on standard medium (4) at 24–25°. For the experiments in Tables 5 and 6, 15 one-day-old virgin females were mated to 15 males for each bottle, passed to fresh medium after 5 days, and eliminated 5 days later. Bottles A–N of Table 6 and 1–17 of Table 5 are combined progeny of both passages. Parents of bottles 18–27 of Table 5 were eliminated after 5 days.

The *y*$^+$ *sc*^{8} c.o. *Y* elements are kept as *Y*^{S}*X*·*Y*^{L}*,In(1)EN+dl49,Y*^{S} *car f v y*·*Y*^{L}*/y*$^+$ *sc*^{8} c.o. *Y* & *C(1)RM,y v f*. The 10 *sc*^{8} c.o. *X* elements we still have are kept as *sc*^{8} c.o. *X/y*$^+$ *bb*$^+$·*Y*^{L} (element N 26 of Table 7) & *C(1)RM,y v f*. Fertility tests were made by combination of X–Y crossovers with the appropriate Y derivatives as indicated in Table 7 and text. Analysis of *bb* loci carried on X–Y crossovers is described in the following section.

RESULTS

Recently, we have shown that rRNA genes of X and Y chromosomes have opposite orientation with respect to their centromeres (4). Therefore a test for X–Y recombination within *bb* during magnification requires not only that the males be *bobbed*, so that magnification will occur, but also that the rDNA of the X chromosome be inverted to permit recovery of the crossover products. The combination used was *In(1)sc*^{8}*,bb*^{1} *cv/Ybb*. In these *bobbed* males magnification of both *bb* loci occurs.

The data on magnification of *Ybb* are shown in Table 1. The *Ybb* chromosomes derived from *sc*^{8}*bb*^{1}*cv/Ybb* fathers, in which magnification occurs, are compared with the same chromosomes derived from *sc*^{8}*bb*$^+$*cv/Ybb* that are wild-type

TABLE 1. *Evidence of magnification of Ybb in In(1)sc*^{8}*bb*^{1}*cv/Ybb males*

Cross	Progeny: Females: cv B^s	Females: cv	Females: y cv	Males: y cv B^s	Males: cv B^s	Males: y cv bb$^+$	Males: y cv bb
In(1)sc^{8}bb$^+$cv/Ybb x sc^{4,8},y cv/sc^{4,8},y cv/B^{s}Y	318	328	—	239	18	—	63
In(1)sc^{8}bb^{1}cv/Ybb x sc^{4,8},y cv/sc^{4,8},y cv/B^{s}Y	316	1	10	202	23	99	49

TABLE 2. *Magnification of bb^1 on $In(1)sc^8bb^1cv$*

Cross	Progeny: Males cv bb	Females y v f	Females y^+ v f
$In(1)sc^8bb^1cv/Ybb^+$ x C(1)RM,y v f/Ybb^-	—	1539	—
$In(1)sc^8bb^1cv/Ybb$ x C(1)RM,y v f/Ybb^-	—*	441	2
$In(1)sc^8bb^{1m1}/Ybb$ x C(1)RM,y v f/Ybb^-	59	1637	10

* Occasionally, bb^{1m}/Ybb^- males can be obtained after only one generation of association of the bb^1 locus with the helper Ybb chromosome. A larger scale experiment identical to this, which shows the point, is reported in Table 5.

TABLE 3. *Estimation of magnification frequency of bb^1 locus on sc^8bb^1cv chromosome after one generation with helper Ybb chromosome*

Cross	Relevant female progeny*: B^S	Relevant female progeny*: Round eyed	Frequency of round-eyed females (%)
$In(1)sc^8bb^1cv/Ybb$ x $sc^{4,8}$,y cv/$sc^{4,8}$,y cv/B^SY	1381	1	0.07†
$In(1)sc^8bb^1cv/Ybb$ x y w bb^1/y w bb^1/B^SY	1266	13	1.02‡

* For simplicity, male progeny and those females receiving two Xs from the mother and a Y from the father are omitted.

† After the experiment reported in Table 5, the frequency of magnification of bb^1 after one step is slightly higher (0.16%) but it is more accurate.

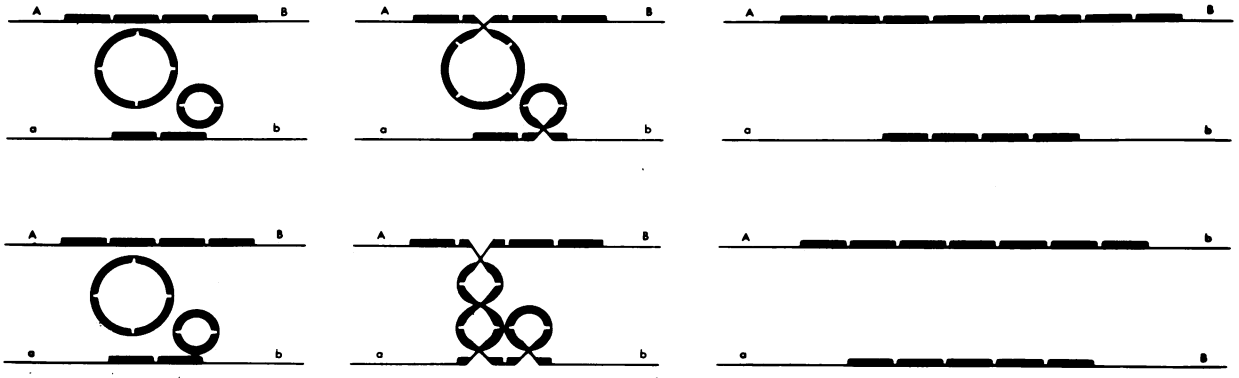
‡ Such frequency, referring to bb^{1m1}/bb^1 individuals, decreased by that given above in this table and referring to bb^{1m1}/bb^0 individuals, gives the frequency of formation of bobbed deficiency-sensitive alleles (bb^{ds}) out of starting bb^1 loci. One has to note that such frequency is only a rough estimate. This because in one case it comes from the evaluation of only one individual, and also because some round-eyed females might be due to nondisjunction of male X and Y chromosomes. Nondisjunction of In(1)sc^8/Y combinations was, however, estimated to be about 0.04% (4).

for *bobbed*, and hence do not show magnification. The comparison is made in the same genetic background, i.e., in combination with bb^0. Magnification is evidenced by the appearance of *y cv* males ($In(1)sc^{4L,8R}$,*y cv/Ybb*) of bb^+ phenotype, and by the higher proportion of *y cv* males from sc^8bb^1cv/Ybb than from sc^8bb^+cv/Ybb fathers.

To detect magnification of bb^1 on the $In(1)sc^8$ chromosome, the locus was maintained for two successive generations in the presence of a helper *bb* locus as described (3). $In(1)sc^8bb^1cv/Ybb^+$ males were crossed to $C(1)RM/Ybb$ females, and the resulting $In(1)sc^8bb^1cv/Ybb$, phenotypically *bobbed*, males were again crossed to $C(1)RM/Ybb$. The sons of this last cross are indicated as $In(1)sc^8bb^{1m1}cv/Ybb$ and are still phenotypically *bobbed*. The bb^1 locus coming from such males will have spent two generations in association with helper *Ybb* under magnifying conditions. Magnification of bb^1 is evidenced by the appearance of $sc^8bb^{1m}cv/Ybb^-$ individuals (where the Ybb^- chromosome has no functional rDNA) after the cross indicated in the third line of Table 2. Before bb^1 magnification the bb^1/Ybb^- combination was absolutely lethal (first line of Table 2).

As observed for other bb^1 loci (3), magnification of bb^1 is regularly detectable after two generations of association with helper. Some magnification of bb^1 occurs after one such generation (3, 5). This result is shown in Table 5, where a large-scale experiment similar to that in Table 2 is reported. Better evidence for this point can be obtained by crossing $In(1)sc^8bb^1$-cv/Ybb males in such a way that the outgoing $In(1)sc^8bb^{1m1}cv$ chromosome is combined with a chromosome carrying some genes for rRNA, such as another bb^1 locus (*y w* bb^1). Since genes for rRNA have an additive effect (6), this test is less restrictive than the combination with Ybb^- or $sc^{4L,8R}$. The results of such a test are shown in Table 3. $In(1)sc^8bb^1cv/Ybb$ males were crossed to *y w* bb^1/*y w* bb^1/B^SY females. For comparison, the same males were crossed to $In(1)sc^{4L,8R}$,*y cv*/$In(1)sc^{4L,8R}$,*y cv*/B^SY females. The appearance of round-eyed females from such crosses indicates magnification of bb^1.

The *cv bb* males from the crosses in Table 2 might be due



FIGS. 1 and 2. (1) (*Top*) Diagram illustrating how extra copies of rDNA could be integrated during magnification of two *bobbed* loci. Each *bb* locus (one with two and one with four genes in this diagram) is supposed here to produce one extra copy of rDNA having its same length (but see ref. 3); this is successively integrated within the same locus from which it is derived.

(2) (*Bottom*) The same situation is illustrated as that shown in Fig. 1, but here one of the extra copies of rDNA is supposed to be simultaneously integrated into both chromosomal *bb* loci. The event leads to recombination of outside markers.

to nondisjunction of the sex chromosomes in males. The point was tested by crossing $In(1)sc^{8}bb^{lm2}cv/Ybb^{-}$ males to $C(1)DX,y\ f/B^{s}Y$ females, where the compound X carries no rDNA (7, 8). The presence of extra Y chromosomes in the males (e.g., magnified *Ybb*) should lead to the appearance of *y f* females. No such females appeared. The test was repeated after further magnification steps, always with negative results.

Table 4 gives the results of experiments in which bb^{lm} was further maintained under magnifying conditions. rDNA magnification, which is a step process, continues until the $sc^{8}bb^{lm}/Ybb^{-}$ individuals are phenotypically wild type.

In the first crosses made, where magnification occurred in sc^{8}/Y males, we immediately noted a high frequency of X–Y recombinants, indicated by the appearance of y^{+} females (Tables 2 and 4). If crossing-over between the Y and sc^{8} chromosomes occurs at the *bb* locus, one of the expected products is a $y^{+}\cdot Y^{L}$ element (Fig. 3), whether the process is meiotic or mitotic (9). We quantified these observations in two large-scale experiments. Table 5 shows the results of the cross between $In(1)sc^{8}bb^{1}cv/Ybb$ males and $C(1)RM,y\ v\ f/Ybb^{-}$ females, and Table 6 shows those of crossing the same males with females homozygous for the $XY^{L}\cdot Y^{S},y^{2}\ su(w^{a})w^{a}$ chromosome. In this latter case, both of the expected crossovers can be recovered. $y^{+}\cdot Y^{L}$ elements (Fig. 3) are revealed as y^{+} males, while $Y^{S}X\cdot$ elements give *yellow* females (9). The majority of X–Y recombinants obtained from these experiments have been characterized for their effective chromosomal composition and their *bb* loci. Some results are reported in Table 7. Of the 38 analyzed $y^{+}sc^{8}$ c.o. *Y* elements, 37 are of the $y^{+}\cdot Y^{L}$ type, thus confirming that the Y^{S} arm, which carries *bb*, was the exchange partner with the inverted X (4, 9). Of the sc^{8} c.o. *X* elements (Table 6), 18 out of 22 were analyzed. All of them gave no viable males when combined with a normal Y chromosome, but gave viable males in combination with $y^{+}bb^{+}\cdot Y^{L}$ element N 26 (Table 7). This is consistent with the idea that sc^{8} c.o. *X* and $y^{+}sc^{8}$ c.o. *Y* elements are reciprocal products. However, in the combinations of sc^{8} c.o. *X* elements with the $y^{+}bb^{+}\cdot Y^{L}$ element N 26, 8 out of 18 males analyzed were sterile. The 10 fertile cases are $Y^{S}X\cdot$ elements missing the y^{+} bearing tip of the X, as expected. Lindsley (9) also observed that some presumed $Y^{S}X\cdot$ elements were sterile in association with Y^{L}.

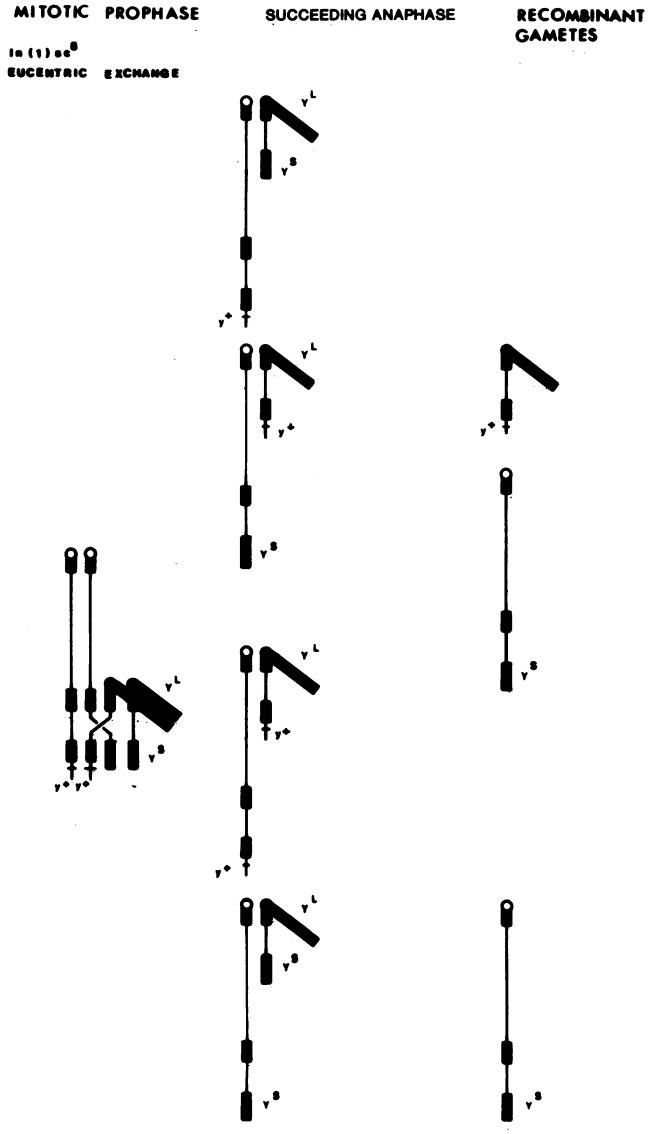


FIG. 3. Diagram showing recombination at *bb* loci of the *Y* and of the inverted *X,In(1)sc*^{8}, chromosome. *bb* Loci have the same sense with respect to the centromere in this case (4). The event is supposed here to be mitotic. A meiotic event of this type will generate the same recombinants. The figure is redrawn from Lindsley (9).

The *bb* loci of crossover elements were classified in the following categories after combining each crossover with a *bb*-, a bb^{-1}, and a bb^{0}-bearing chromosome:

(a) bb^{+}. Elements that give a wild, or almost wild, phenotype with all three tester chromosomes (and in particular with bb^{0}).

(b) *bb*. Elements that give a *bobbed* phenotype in combination with bb^{1} and bb^{0}. This category is the most arbitrary, since the *bobbed* phenotype of the different elements varied in intensity.

(c) bb^{ds}. Elements that give *bobbed* progeny in combination with the bb^{1} tester chromosome, but no progeny in combination with bb^{0} (10).

TABLE 4. *Successive steps of magnification of bb*^{1}*locus on In(1)sc*^{8}*bb*^{1}*cv chromosome*

Parent males*	Phenotype of males	Progeny: Males bb^{+}	Progeny: Males bb	Progeny: Females y v f	Progeny: Females y^{+} v f
In(1)sc^{8}bb^{lm2}/Ybb^{-}	Strong bb	—	230	591	2
In(1)sc^{8}bb^{lm2}/Ybb^{-}†	Strong bb	—	176	788	1
In(1)sc^{8}bb^{lm3}/Ybb^{-}	bb	26	349	1463	2
In(1)sc^{8}bb^{lm3}/Ybb^{-}†	bb	23	266	726	1
In(1)sc^{8}bb^{lm4}/Ybb^{-}	bb	29	103	348	—
In(1)sc^{8}bb^{lm5}/Ybb^{-}	Random	113‡		435	—
In(1)sc^{8}bb^{lm7}/Ybb^{-}	Random‡	Not counted		19,472	3

* The indicated males were crossed to C(1)RM,y v f/Ybb^{-} females.

† Each cross was made in duplicate from two lines independently obtained.

‡ The majority of individuals were almost bb^{+}. Classification, however, cannot be very accurate when bb approaches wild type due to scute effect on bristles.

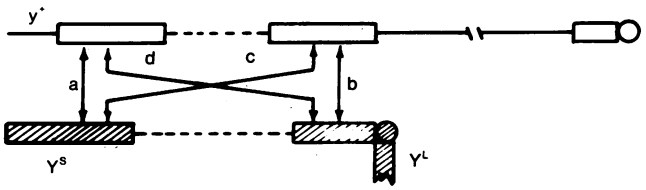


FIG. 4. Diagram illustrating eucentric pairing between the short arm of the *Y* and the inverted heterochromatin of *In(1)sc*^{s} chromosome. *bb* Loci are indicated by the *dashed line*. The possible exchanges indicated as (*a*),(*b*),(*c*), and (*d*) could all justify the appearence of $y^{+}.Y^{L}$ and $Y^{s}X.$ crossovers, besides recombination at the *bb* level.

(**d**) bb^{1} Elements that give no progeny with either bb^{1} or bb^{0}. Such elements give a *bobbed* phenotype with the *bb* tester.

The results of such tests on $y^{+}\cdot Y^{L}$ crossovers are presented in Table 7. Among $Y^{s}X\cdot$ crossovers (data omitted) examples were also found in each of the preceding four categories.

DISCUSSION

The frequency of recombination between the X and Y chromosomes during rDNA magnification is about 0.3% (0.33% in the cross used for Table 6, and 0.27% in that of Table 5); whereas in $sc^{s}bb^{1}cv/Ybb^{+}$ males, where magnification does not occur, it is about 0.01% (4), and it is about 0.015% in $sc^{s}bb^{1m7}cv/Ybb^{-}$ males (Table 4) where magnification is almost complete. We can therefore calculate a 20- to 30-fold increase of recombination between X and Y chromosomes during rDNA magnification.

In agreement with previous experiments (4, 9) our results show exchange between the short arm of the Y and the in-

TABLE 5. *Outcome of the cross between In(1)sc*^{s}*bb*^{1}*cv/Ybb males and C(1)RM,y v f/Ybb*$^{-}$*females*

Bottle number	Males* cv bb	Females y v f	Females y+ v f	Designation of y+ bearing element
1	—	496	—	
2	2	394	3	2 lost, 36
3	—	430	2	48, 49
4	1	313	—	
5	—	520	3	45, 46, 47
6	—	654	1	lost
7	—	608	1	50
8	2	653	3	one lost, 34, 53
9	1	554	—	
10	—	555	3	35, 51, 52
11	1	524	4	39, 56, 57, 58
12	—	414	—	
13	1	499	1	40
14	—	452	1	59
15	—	383	—	
16	2	332	—	
17	1	319	1	54
18	1	192	—	
19	—	163	—	
20	—	168	1	41
21	—	147	—	
22	—	206	—	
23	3	172	1	42
24	1	119	—	
25	—	199	—	
26	—	162	1	43
27	—	169	1	44
	16	9797	27	

* Also, 3 y v f males were obtained. They are due to detachment of compound Xs after crossing over with the Ybb^{-} chromosome.

TABLE 6. *Outcome of the cross between In(1)sc*^{s}*bb*^{1}*cv/Ybb males and XY*^{L}*.Y*^{s}*,y*^{2} *su(w*^{a}*)w*^{a}*homozygous females*

Bottle	Males y	Males y+	Designation of y+sc^{s}c.o.Y element	Females y+	Females y
A	400	1	A1	466	1
B	450	3	B1, B2, B3	617	1
C	327	4	one lost C1, C2, C3 and C4	430	1
D	580	3	2 lost, D1	735	4
E	673	1	E1	734	1
F	252	—		317	—
G	625	3	one lost, G1, G2	699	3
H	476	1	H1	573	2
I	430	—		603	3
L	460	—		652	—
M	387	1	M1	494	4
N	374	1	N1	583	2
	5434	18		6903	22

TABLE 7. *Analysis of y*$^{+}$*sc*^{s}*c.o.Y elements*

Designation of element	Origin	Fertility with X.Y.s*	Fertility with X	bb Locus
40,47	In(1)sc^{s}bb^{1}/ Ybb	F	S	bb^{+}
39,43,44,46,49,52,53, 54,57				
58,B1,B3,C1,C4,E1, G2,H1 M1	In(1)sc^{s}bb^{1}/ Ybb	F	S	bb
34,41,48,51,59,B2,C3, D1,G1	In(1)sc^{s}bb^{1}/ Ybb	F	S	bb^{ds}
35,36,42,45 50,56‡, A1,N1	In(1)sc^{s}bb^{1}/ Ybb	F	S	bb^{1}
14,19,26	In(1)sc^{s}bb^{1m}/ Ybb^{-}†	F	S	bb^{+}
13,17,21,25,27,29	In(1)sc^{s}bb^{1m}/ Ybb^{-}	F	S	bb
15,24	In(1)sc^{s}bb^{1m}/ Ybb^{-}	F	S	bb^{ds}
12,16,28	In(1)sc^{s}bb^{1m}/ Ybb^{-}	F	S	bb^{1}

* Only elements designated by a number were effectively tested for fertility. Since all but one tested y^{+}-bearing elements resulted to be of the $y^{+}.Y^{L}$ type, fertility tests were not made in the latest experiments.

† The bb^{1m}loci listed in this table were from bb^{1m2} to bb^{1m5}. Males carrying such loci were from bb to almost bb^{+} in phenotype.

‡ Element N 56 is sterile with both X and X.Y^{s} chromosomes.

verted part of the X chromosome, where rDNA is located. Formally, $y^+ \cdot Y^L$ and $Y^S X \cdot$ products could result from exchange between regions neighboring *bb*. Any of the four possible exchanges illustrated in Fig. 4 could account for such elements, besides recombination at the rDNA level. However, we have already shown that $y^+ \cdot Y^L$ elements are formed by exchange within rDNA in sc^s/Ybb^+ combinations (4). Furthermore, if we consider the variation in *bb* among recombinants analyzed in the present paper, we have to conclude that at least some of the exchanges must have occurred at the rDNA level. An example is given by the $y^+ \cdot Y^L$ elements N 15 and 24 (Table 7) which are bb^{ds} derived from $sc^s bb^{lm4}/Ybb^-$. Since the bb^{lm} locus in this case was either *bb* or bb^+, only event (*a*) of Fig. 4 could explain their origin; but this would require the complicated assumptions that the exchange makes the bb^- locus of the Y chromosome operative, and that the activated bb^- locus is bb^{ds} despite the fact that it carries about 80 rRNA genes (11). We can also consider $y^+ \cdot Y^L$ elements N 34,41, etc. of Table 7 that derive from $sc^s bb^1 cv/Ybb$ and are bb^{ds}. Their origin could be explained by events (*b*) or (*c*) in Fig. 4, but only with the hypothesis that the bb^1 locus had magnified to the point of becoming bb^{ds}. Although this event is in fact possible (Table 3), its frequency is about 1% in the progeny of $sc^s bb^1 cv/Ybb$ males, while the frequency of bb^{ds} among $y^+ \cdot Y^L$ elements derived from the same males is estimated to be 24% (Table 7). On the basis of these considerations we think that crossing over at the *bb* level during rDNA magnification is the most direct and complete explanation of our results.

Of the 36 analyzed $y^+ \cdot Y^L$ elements formed during magnification (Tables 5 and 6; 44 reported, 8 lost), at least 32 can be shown to be independent events by their occurrence in separate bottles or by differences in their *bb* loci. The scarcity of clusters suggests that the exchanges are not mitotic (4, 9), a point that is being further tested. Exclusion of mitotic exchange lends strong support to the idea that the increase of rDNA during magnification is caused by extra synthesis. In that case there is no better way to insert the extra copies of rDNA than by crossing-over, as postulated in our model (3). Such a feature may be extended to other cases of extra synthesis, such as rDNA amplification (12–15). It allows parallel evolution of rDNA located in different chromosomes, and it may play a role in maintaining homogeneity of repeated sequences during evolutionary change, the process termed "horizontal evolution" by Brown, Wensink, and Jordan (16).

This work was aided by CNR contract no. 72/0646/04.

1. Ritossa, F. M., Atwood, K. C. & Spiegelman, S. (1966) *Genetics* **54**, 819–834.
2. Ritossa, F. (1968) *Proc. Nat. Acad. Sci. USA* **60**, 509–516.
3. Ritossa, F. (1972) *Nature New Biol.*, **240**, 109–111.
4. Palumbo, G., Caizzi, R. & Ritossa, F. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 1883–1885.
5. Atwood, K. C. (1969) *Genetics* **61**, Suppl. 1, 319–327.
6. Stern, C. (1929) *Biol. Zentralbl.* **49**, 261–290.
7. Lindsley, D. L. & Grell, E. H. (1968) *Carnegie Inst. Wash. Publ.* No. 627.
8. Ritossa, F., Malva, C., Boncinelli, E., Graziani, F. & Polito, L. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 1580–1584.
9. Lindsley, D. L. (1955) *Genetics* **40**, 24–44.
10. Stern, C. & Ogura, S. O. (1931) *Z.i.A.V.* **58**, 81–121.
11. Ritossa, F. (1968) *Proc. Nat. Acad. Sci. USA* **59**, 1124–1131.
12. Brown, D. D. & Dawid, I. B. (1968) *Science* **160**, 272–280.
13. Birnstiel, M. L., Chipchase, M. & Speirs, J. (1971) *Progr. Nucl. Acid Res. Mol. Biol.* **11**, 351–389.
14. Gall, J. G. (1969) *Genetics* **61**, Suppl. 1, 121–132.
15. MacGregor, H. C. (1972) *Biol. Rev.* **47**, 177–210.
16. Brown, D. D., Wensink, P. C. & Jordan, E. (1972) *J. Mol. Biol.* **63**, 57–73.